

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
2 August 2001 (02.08.2001)

PCT

(10) International Publication Number
WO 01/54727 A1

- (51) International Patent Classification⁷: **A61K 45/00**
- (21) International Application Number: PCT/US00/28361
- (22) International Filing Date: 13 October 2000 (13.10.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/159,567 15 October 1999 (15.10.1999) US
- (71) Applicant (for all designated States except US): **THE VAN KAMPEN GROUP, INC.** [US/US]; 1008 Lake Winds Drive, Hoover, AL 35244-3243 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **VAN KAMPEN, Kent, R.** [US/US]; 881 East, 5550 South, Ogden, UT 844506 (US). **EDWARDS, Bobby, G.** [US/US]; 19448 FM 2115, Salado, TX 76571 (US).
- (74) Agents: **CLARK, W. Robinson, H.** et al.; Dorsey & Whitney LLP, 801 Grand Suite 3900, Des Moines, IA 50309 (US).
- (81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TREATMENT OF DERMAL TUMORS, WARTS, AND VIRAL INFECTIONS USING HEAT-KILLED *P. ACNES*

(57) Abstract: Heat-killed, terminally sterilized saline suspensions of *Propionibacterium acnes*, *Propionibacterium avidum*, *Propionibacterium lymphophilum*, *Propionibacterium granulosum*, *Corynebacterium parvum*, and *Arachnia propionica* are effective in treating viral infections of the respiratory tract in humans, and to induce the regression of dermal tumors and warts in humans. The potency of a saline suspension of heat-killed, terminally sterilized saline suspension of *Propionibacterium acnes* (*P. acnes*) was demonstrated through a laboratory animal challenge model. The *P. acnes* product is administered orally for the purpose of preventing or treating viral infections of the respiratory tract in man. The *P. acnes* preparation is intralesionally administered into dermal tumors, warts such as plantar warts, or other warts in people caused by the human papilloma virus, to cause regression of such dermal tumors and warts. The subcutaneous route of administration of the *P. acnes* product causes a systemic reaction that causes long-term warts to completely regress. Anesthetics such as Lidocaine may be added to the *P. acnes* product to prevent pain upon injection of this immune modulating preparation, while retaining the potency of the *P. acnes* product. Dose ranges have been established for the oral administration of the *P. acnes* product to treat viral infections, and for the subcutaneous and intralesional administration of the *P. acnes* product to treat dermal tumors and warts.

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1 **THE TREATMENT OF DERMAL TUMORS, WARTS, AND VIRAL INFECTIONS**
2 **OF THE RESPIRATORY TRACT IN HUMANS USING HEAT-KILLED *P. ACNES***

3 **Field of the Invention**

4 The present invention relates to methods to treat viral infections, dermal tumors, and warts
5 in humans using heat-killed bacterial compositions. Specifically, it relates to the subcutaneous or
6 intralesional administration of heat-killed *Propionibacterium acnes* (*P. acnes*), to treat dermal
7 tumors and warts, and to the oral administration of heat-killed *P. acnes* to treat virus induced
8 infections of the respiratory tract in humans.

9 **Background of the Invention**

10 The maintenance of a healthy and competent immune system is a prerequisite for resistance
11 to and elimination of infectious and neoplastic diseases. Bacteria and their derivatives were among
12 the first substances to be recognized as immunostimulators and are used as adjuvants in vaccines to
13 boost the humoral immune response (*e.g.*, complete Freund's adjuvant). Bacteria have also been
14 used as non-specific enhancers of the immune system to increase resistance and rejection of
15 cancers, parasites, and infectious organisms.

16 Gram positive, whole-cell bacteria such as *Propionibacterium acnes*, *Propionibacterium*
17 *avidum*, *Propionibacterium lymphophilum*, *Propionibacterium granulosum*,
18 *Corynebacterium parvum* and *Arachnia propionica*, when inactivated have been shown to be
19 potent non-specific immune stimulants in animals and humans. Specifically *Propionibacterium*
20 *acnes* (*P. acnes*) has been shown to stimulate antineoplastic activity, adjuvant activity, antiviral
21 activity, antibacterial activity, and to stimulate hematopoiesis.

22 Preparations of *P. Acnes* have been shown to act as non-specific stimulators of
23 immunogenic responsiveness in vivo. *P. Acnes* is known to act by stimulating macrophages and
24 neutrophils, initiating endogenous production of lymphokines (including IL-2 and various
25 interferons), and enhancing killer cell activity. The intranasal inoculation of mice with *P. acnes* has
26 been shown to activate pulmonary macrophages (Jackson RA, et al., *J Leukoc. Biol.*, 40(5):575-
27 87, 1986). At the cellular level, *P. acnes* acts upon monocytes and lymphocytes and improves the
28 functional interaction between these cells (M.T. Scott, *Cell Immunol.*, 17:141, 1975).

29 *P. acnes* also functions as an immune adjuvant to weakly antigenic substances. These
30 properties, while not completely understood, play an important role in regulation of the immune

1 response. One mode of the interaction of inactivated *P. acnes* with the immune system is through
2 its stimulation of the reticuloendothelial system (RES), *i.e.* liver, spleen, lymph nodes, lungs, and
3 bone marrow (C. Adlam, and M.T. Scott, *J. Med Microbiol*, 6:621 (1973), N.H. McBride *et al.*,
4 *Cell Immunol.*, 7:290 (1973)).

5 This activity elicits enhanced resistance to bacterial and viral infections, and also to certain
6 tumors. The mode of action appears to be the activation of macrophages followed by the
7 recruitment of lymphocytes. The particulate nature of *P. acnes* appears important for macrophage
8 activation. Unlike some synthetic biological response modifiers (BRM's), bacteria *in vivo* are fully
9 degraded and catabolized in the body without the formation and excretion of toxic metabolites or
10 retention of residues. This has obvious therapeutic advantages for *P. acnes*. and contributes to the
11 therapeutic and prophylactic use of *P. acnes* against infectious diseases.

12 In animals, stimulation of the immune system results in short term protection against infection
13 with certain viruses and bacteria. Used therapeutically in animals with chronic skin and respiratory
14 disease, *P. acnes* shortens the course of the disease.

15 The anti-tumor activity of *P. acnes* has been studied in mice and other animals. Tumor cells
16 injected into Balb/c mice together with heat-killed *P. acnes* cells were rendered nontumorigenic
17 (Murano EA, *et al*, *Cancer Immunol Immunother*, 29(1):7-16, 1989). The preventive effect of
18 *P. acnes* on metastasis in mice rendered tolerant to tumor-associated transplantation antigens
19 (TATA) has been detailed (Fujiwara H, *et al* , *Gann*, 71(5):692-8, 1980). Heat-killed
20 suspensions of several *P. acnes* strains were prepared and studied for their protective activity
21 against viral infections in mice and for their immunomodulating properties (Zgorniak-Nowosielska I,
22 *et al*, *Arch Immunol Ther Exp (Warsz)*, 37(3-4):431-42, 1989).

23 There has been considerable data collected on the use of *P. Acnes* in domestic animals. In
24 a randomized study conducted for the treatment of equine respiratory disease (ERDC), complete
25 recovery within a 14 day period was observed in horses treated intravenously with *P. acnes* (D. R.
26 Evans *et al.*, *Equine Practice*, 10:17, 1988; C.D. Vail *et al.*, *Vet. Review*, Nov/Dec: 399, 1990).
27 Additionally, inactivated *P. acnes* has also been shown to be a biological response modifier for
28 treatment of non-specific respiratory diseases in horses where upon administration of *P. acnes* it

1 was shown that CD4⁺ lymphocyte expression and lymphokine activated killer cell (LAK) activity
2 increased (Flaminio MJ, *et al*, *Vet Immunol Immunopathol*, 63(4):303-15, 1998).

3 In a randomized, double blinded, placebo controlled study, dogs with a significant skin
4 disease (chronic recurrent pyoderma) were treated with antibiotics plus *P. acnes* with significant
5 improvement or complete remission of the lesions (A. Becker *et al.*, *J. Vet Intern. Med.* 13:26
6 (1989)).

7 *P. acnes* has been extensively used as a veterinary therapeutic in cattle with papilloma
8 (warts) where the warts had been intralesionally injected with *P. acnes* (H. Hall *et al.*, *Therapeutic*
9 *Immunology*, 1:319, 1994). While, lesions in the control group which were injected with saline
10 showed no regressions at the end of 16 weeks, 100% of the injected lesions in the treatment group
11 had completely regressed at the end of 16 weeks.

12 Use of *P. acnes* in humans has, in general been limited to treatment of neoplastic diseases
13 and pleural effusions with some limited success. Additionally, *P. acnes* has been administered
14 orally in the rations of food production animals to promote better health through cell-mediated
15 immunity and weight gain (U.S. Patent Application Serial No. 08/912,026). It has been used
16 experimentally in people to treat various cancers, plural effusion and chronic obstructive pulmonary
17 disease. It has been used experimentally as an adjuvant with vaccines.

18 Based on these findings, a veterinary preparation of *P. acnes* was used as an injectable
19 therapeutic agent against plantar warts caused by the human papilloma virus. However, significant
20 pain upon injection was observed caused due to the alcohol content of the preparation. Thus, a
21 preparation of *P. acnes* is needed that causes the regression of warts and dermal tumors in humans,
22 but which may be administered without undue pain or harm to the patient. Additionally, this
23 preparation must be administered via a route that allows regression of the warts while minimizing
24 pain to the patient .

25 Although *P. acnes* has been used to treat respiratory diseases in horses and cattle, the oral
26 administration of *P. acnes* with efficacy in humans has not been previously demonstrated. There is

1 a need for a *P. acnes* preparation that can be safely administered to humans for the treatment of
2 viral infections of the respiratory tract.

3 *P. acnes* preparations have been administered primarily through intravenous,
4 intraperitoneal, or intrathoracic routes. However, they may also be administered orally,
5 subcutaneously, or intralesionally depending on the type of infection and the determined dosage. *P.*
6 *acnes* has been used at higher dose levels in experimental animals to study the release of nitric oxide
7 by cells or the liver and other body tissues, and has been combined with vaccines as an adjuvant for
8 subcutaneous or intramuscular injection. Ethanol-saline suspended preparations of heat-killed *P.*
9 *acnes* for veterinary use in treating pyoderma, a bacterial infection in dogs, and respiratory
10 infections in horses have been used. However, these preparations had to be administered
11 intravenously in order to be efficacious. In another case, a feed additive consisting of dried *P.*
12 *acnes* mixed with feed rations was given to baby pigs which subsequently exhibited decreased
13 mortality, increased weight gain and feed conversion. However, optimization of the route of
14 administration for the treatment of dermal warts, tumors, and viral infections of the respiratory tract
15 in humans has not hitherto been conducted.

16 In order to efficaciously administer the *P. acnes* preparation, an optimal mode of
17 inactivation of the *P. acnes* preparation is also needed. Although, suspending the *P. acnes* in an
18 ethanol-saline suspension causes inactivation of *P. acnes*, the presence of ethanol causes discomfort
19 in humans. Thus, there is a need to safely and adequately inactivate the *P. acnes* without any undue
20 loss in activity. Heat-killing is an efficacious method of inactivating *P. acnes*. However, there is a
21 need to develop a method of heat-killing that adequately inactivate the *P. acnes* while maintaining
22 desired levels of activity.

23 **Summary of the Invention**

24 This is an invention to induce regression of a virally induced dermal tumor, especially plantar
25 warts for which painful surgical removal or chemical burning are the most common methods of
26 removal. These alternate methods cause severe pain and limit mobility to a majority of patients
27 receiving these treatments. It is also an invention to treat and hasten recovery from virally induced

1 infection of the respiratory tract using autoclaved *P. acnes* through a novel route of administration,
2 previously not demonstrated in man, that of oral administration.

3 This invention also relates to the preparation of an alcohol-free, terminally sterilized saline-
4 suspended *P. acnes* product that causes the regression of dermal tumors, and plantar warts in
5 humans. Terminal sterilization may be conducted through the process of autoclaving. In another
6 embodiment of the product, an anesthetic such as lidocaine is added to the *P. acnes* product. The
7 invention also relates to a novel intralesional administration of the *P. acnes* product into plantar
8 warts, or other warts caused by the human papilloma virus causing regression of such warts, and the
9 subcutaneous administration of the *P. acnes* product resulting in a systemic regression of warts.

10 **Detailed Description of the Invention**

11 This invention relates to the preparation, administration, and use of an inactivated bacterial
12 product to induce regression of virally induced dermal tumors and warts, and to effectively treat
13 virally induced infections of the respiratory tract. The warts may be plantar, genital, or surface
14 warts anywhere on the skin or mucosal surface of the body, or those caused by the human
15 papilloma virus.

16 The bacteria used for practicing the invention may be selected from the group consisting of
17 *Propionibacterium acnes*, *Propionibacterium avidum*, *Propionibacterium lymphophilum*,
18 *Propionibacterium granulosum*, *Cornynebacterium parvum* or *Arachnia propionica*.
19 Preferably, the bacteria used for practicing the invention are selected from the *Propionibacterium*
20 family. Most preferably, the bacteria used for practicing the invention is *Propionibacterium acnes*
21 (*P. acnes*). Thus, *P. acnes* will be the bacterium referred to throughout the description, although
22 any of the bacterial species claimed can be substituted. However, the statements contained in this
23 description should apply to each of the bacteria claimed unless otherwise indicated, since all of the
24 claimed bacteria are expected to have the same results due to their taxonomic similarity. Although it
25 is now recognized that *Cornynebacterium parvum* (*C. parvum*) is thought to be synonymous with
26 *P. acnes*, it has been included in the list due to the use of the name that still exists in the art.

1 In the present invention, a method for preparing a saline suspension of heat-killed *P. acnes*
2 with demonstration of potency through a laboratory animal challenge model is disclosed. It has
3 been determined that heat-killing, which usually destroys or alters the antigens needed to stimulate
4 the immune responses, does not destroy the potency of the autoclaved *P. acnes* product.
5 Furthermore, as shown in laboratory animal potency tests, the addition of an anesthetic such as
6 lidocaine to the autoclaved *P. acnes* product does not destroy the potency of the *P. acnes* product.

7 *P. acnes* is known to be commercially available in forms such as an injectable solution (e.g.,
8 ImmunoRegulin® or EqStim® by Neogen Corp. (Lansing, MI)), but it may also be isolated and
9 cultured by known, standard bacterial procedures or obtained from national culture collections.
10 The bacteria used were obtained from ImmunoVet Corp. (Tampa, FL) who produced them under
11 U.S.D.A. Product Code 9350.00. The bacteria may also be obtained from Neogen Corp.
12 (Lansing, MI). The bacteria may be provided wet or dry. A dry form may be prepared by
13 standard drying methods known to a person skilled in the art. such as freeze-drying or evaporation.

14 *P. acnes* may be manufactured by laboratory processes known in the art. *P. acnes* may be
15 isolated and cultured by standard cell culture methods. The *P. acnes* product is prepared by
16 culturing *P. acnes* on solid or in liquid media at a temperature of 36 °C +/- 2 °C for 24 to 192
17 hours, depending on the culture conditions. *P. acnes* may be grown on plates, e.g., agar plates
18 containing various nutrients, or in bioreactors. The bioreactors include stationary culture flasks,
19 shaker flasks, standard fermentors, hollow fiber reactors, perfusion reactors, plug flow reactors,
20 etc., containing a fermentation broth with nutrients in dissolved form such as glucose, starches,
21 tryptic soy broth, hormones, coenzymes, and optionally serum. *P. acnes* is then collected using
22 standard separation methods such as centrifugation, and tested for purity by immunofluorescence
23 or biochemical testing.

24 The *P. acnes* is dried while subjected to heat sufficient to inactivate and kill it. Heat-killing
25 is preferably conducted by heating the *P. acnes* in a water bath at 74 °C to 90 °C for 60 to 90
26 minutes. The *P. acnes* is then weighed and suspended in a sterile saline solution at a concentration
27 of .005 to 10 mg/ml. The exact concentration is determined by the proposed use of the product, be
28 it the treatment of warts or viral infections of the respiratory tract. The saline solution comprises

1 sodium chloride in a buffer selected from the group consisting of alkaline metal phosphate or citrate
2 buffers, such as sodium phosphate, potassium phosphate, sodium citrate, and potassium citrate, or
3 sodium chloride in dI water. Preferably, the concentration of the sodium chloride is 0.85 % w/v,
4 more preferably the concentration of the sodium chloride is 0.9 % w/v.

5 Optionally, the *P. acnes* may be mixed with carriers and fillers, and brought into the form of
6 a therapeutically enteric pharmaceutical composition. Suitable carriers are sugars including but not
7 limited to lactose, saccharose, mannitol, or sorbitol; cellulose preparations, amino acids such as
8 glycine, binders such as starch pastes that use corn, wheat, rice or potato starch, gelatine,
9 methylcellulose, hydroxypropylmethylcellulose, and sodium carboxymethylcellulose.

10 Optionally, an anesthetic may be added to the *P. acnes* product to induce local anesthesia
11 when administered to the patient. Local anesthetics are drugs that block the generation and
12 propagation of impulses in excitable tissues, most notably the spinal cord, spinal nerve roots, and
13 peripheral nerves, but also skeletal muscle, cardiac muscle, and the brain. Preferably, the anesthetic
14 is chosen from the group consisting of aminoamides, such as lidocaine (xylocaine), and aminoesters
15 such as 2-Chloroprocaine. Preferably, the local anesthetic is lidocaine (xylocaine). Preferably, the
16 anesthetic is added to the *P. acnes* preparation to make a final concentration of 0.25 % to 5.0 %
17 v/v, more preferably at a final concentration of 0.5% to 2.5% v/v, and most preferably at a final
18 concentration of 1% to 2% v/v.

19 The *P. acnes* may be lyophilized at any step in the preparation process depending on
20 whether the final pharmaceutical formulation is to be stored as a liquid with stabilizing fillers, or as a
21 lyophilized solid.

22 Once the *P. acnes* product is in the final vial, it is terminally sterilized by heating to 121 °C,
23 for 20 minutes, at a pressure of 15 psi.

24 The *P. acnes* product may be tested for potency using standard animal inoculation tests
25 which consists of pre-inoculating the animal with the product, followed by a lethal challenge of a
26 known bacterial pathogen at 1- 7 days which kills at least 75% of the non-inoculated control
27 animals. The dosage units tested are equivalent to 10^9 - 10^{13} *P. acnes*, preferably 10^{10} - 10^{12} *P.*

1 *acnes*. Lidocaine (xylocaine) is added at a dosage that does not affect the potency of the
2 formulation. The laboratory animal potency tests demonstrated that this local anesthetic does not
3 adversely affect the potency of the product.

4 In the present invention, the autoclaved *P. acnes* product is administered intralesionally or
5 subcutaneously to cause the regression of plantar warts in humans. The *P. acnes* product retains
6 activity once autoclaved and once injected, and may be used with or without the addition of an
7 anesthetic. However, the novel addition of anesthetics like lidocaine to this immune modulating
8 preparation of *P. acnes* retains the potency of the *P. acnes* while preventing pain upon injection.
9 The warts may be plantar, genital, or surface warts located anywhere on the skin or mucosal
10 surface of the body. The subcutaneous route of administration of the *P. acnes* product causes a
11 systemic reaction that causes long-term warts to completely regress. Specifically, the subcutaneous
12 injection of the product into the arm induces the regression of warts located on the hands or feet of
13 the patients receiving the injection. Thus, it has been determined that at doses prescribed for
14 intralesional injections, subcutaneous injection may also be effective in causing a systemic regression
15 of the warts. Multiple injections may be made intralesionally or subcutaneously for the purpose of
16 treating plantar warts. Repeated doses in animals or humans have not resulted in any cumulative
17 toxicity. Since the plantar warts are the most difficult variety of the human papilloma to treat,
18 multiple injections may be required over time. However, a single injection may cause regression of
19 the wart. For the regression of warts, the *P. acnes* is administered at a dose of .001 to 5 mg per
20 dosage, preferably at a dose of .005 to 2.5 mg per dosage, and more preferably at a dose of .01 to
21 1 mg per dosage.

22 The *P. acnes* product may also be used to treat chronic complications of the respiratory
23 tract due to viral or bacterial infections where symptomatic coughs are persistent. The *P. acnes*
24 product is orally administered as a treatment for acute or subacute viral infections of the respiratory
25 tract in people, at a dose range of 0.1 to 10 mg, and more preferably at a dose range of 0.5 to 5
26 mg. Oral administration of the heat killed, terminally sterilized *P. acnes* saline product will hasten
27 recovery from virally induced infections of the upper and lower respiratory tract. Optionally, an
28 FDA approved natural or synthetic flavoring is added to the final product to make the administered

1 product more palatable. The FDA approved natural flavorings are listed in the Code of Federal
2 Regulations, 21 CFR 172.510. The synthetic flavorings are listed in 21 CFR 172.515.

3
4 The complete disclosure of all patents, patent documents, and publications cited herein are
5 incorporated by reference. The detailed descriptions and examples herein have been given for
6 clarity of understanding only. No unnecessary limitations are to be understood therefrom. The
7 invention is not limited to the exact details shown and described, for variations obvious to one
8 skilled in the art will be included within the invention defined by the claims.

9 **Example 1**

10 **Treatment of sore throat, ear ache and cough by oral administration of autoclaved, heat-** 11 **killed *P. acnes*.**

12 A sterile saline suspension of non-viable *P. acnes*, terminally autoclaved for 15 minutes at
13 15 psi, was orally administered to patients to impede the advancing clinical signs of upper and lower
14 respiratory tract infections, clinically manifested as sore throat, ear ache, and cough.

15 *P. acnes* was orally administered to two patients to treat the onset of symptoms of a sore
16 throat and ear inflammation. In each case, the treatment consisted of 2 ml of a saline suspension of
17 non-viable, heat-killed and terminally sterilized *P. acnes* at a concentration of 0.4 mg per ml. The
18 success of the treatment demonstrates the efficacy of orally administer *P. acnes* to minimize
19 infections of the respiratory tract in humans. Either one dose or more may be used safely to treat
20 the symptoms of disease.

21 The first patient was a 60-year old Caucasian male weighing 190 pounds. The patient was
22 treated with the suspension on two separate occasions. The patient had symptoms of a sore throat
23 and ear inflammation. The treatment was administered orally. The material was held at the back of
24 the mouth for about 1 minute before swallowing. In about 8 to 12 hours following the treatment, the
25 patient felt somewhat flushed, a symptom that could be related to the infection or to
26 immunostimulation. Within 24 hours, the onset of the sore throat and the ear infection diminished.

1 Within 2 days, the patient was healthy with no remaining symptoms of the sore throat and ear
2 infection.

3 In October, 1998, the patient displayed symptoms of sneezing, coughing, nasal discharge,
4 sore throat, and aching ears. The treatment was administered orally. The material was held at the
5 back of the mouth for about 1 minute before swallowing. Within the following 24 hour period, the
6 patient again noted a slight febrile response. A second dose, similar to the first dose, was
7 administered twenty-four hours following the first dose. No febrile response was observed after
8 this administration. No symptoms of inflammation of the throat and ears were observed after the
9 first day. However, mild coughing and nasal discharge continued on the second day. On the third
10 day, the symptoms began to abate and on the fourth day, they were entirely gone.

11 The second patient was a 32-year old Caucasian female weighing about 140 pounds. The
12 patient had a hoarse voice and complained of an ear ache and sore throat. She was given a similar
13 suspension in the same amount as mentioned above. She did not express any adverse reactions or
14 any symptoms other than those relating to her upper respiratory tract infection. The day following
15 treatment, her throat felt better and within two days thereafter, she was again healthy.

16 This finding demonstrates the efficacy of orally administer *P. acnes* to minimize infections of
17 the respiratory tract in people. Either one dose or more may be used safely to treat the symptoms
18 of disease.

19 Example 2

20 Preparation of *P. acnes*.

21 *P. acnes*, grown on solid or in liquid media at a temperature of 36 ° C for 7 days is
22 separated, tested for purity (by immunofluorescence) and/or biochemical testing, dried while
23 subjected to heat sufficient to kill it, weighed, and suspended in sterile saline at the desired
24 concentration. In the final vial, the product is terminally sterilized for 20 minutes at 15 psi. Or the
25 product can be modified by (through sterile filling) the addition of lidocaine at the desired
26 concentration to induce local anesthesia when injected. The product is then tested for potency using
27 the laboratory animal inoculation test which consists of pre-inoculation with the product and

1 followed several days later by a lethal challenge of a known bacterial pathogen which kills at least
2 75% of the non-inoculated control animals.

3 **Example 3**

4 **Evaluation of the safety of injecting heat-killed *P. acnes* into volunteers with plantar** 5 **warts.**

6 The purpose of this Phase I Safety Study was to evaluate the safety of injecting heat-killed,
7 *P. acnes* into volunteers with plantar warts. Two routes of administration were utilized, intralesional
8 and subcutaneous. Two dose levels of experimental product (0.1 mg and 0.2 mg.) were injected.
9 The control group was injected intralesionally with sterile saline at a volume consistent with the 0.2
10 mg amount of *P. acnes*. Safety parameters were assessed by changes or lack of changes in
11 physical, hematologic, biochemical, and immunologic parameters. The lot # of the Test Article was
12 022497 and the Placebo was lot #KVK794220. Concentration of *P. acnes* was 0.4 mg. per
13 milliliter. In order to test for reactions resulting in repeated injections, the volunteers received a
14 series of three injections at intervals of one week. The patients were randomized upon entry to the
15 study and the study was placebo controlled and blinded to the patient, but not to the investigator.
16 The patients were monitored for four weeks following the initial injection.

17 Anticipated reactions were monitored along with changes in the blood cells, blood
18 chemistry and in the urine. Provisions were in place to focus on any unexpected adverse reactions.
19 The various systemic events monitored included elevated temperature, headache, muscle pain,
20 weakness, chills, nausea, and at the injection site, pain, swelling, redness and discoloration. These
21 are reported on each patient, grouped by treatment and recorded by severity. A summary by
22 treatment groups of the anticipated reactions by number of patients and severity is provided.
23 Separate summary sheets of the observed hematological, chemical and urine changes are also
24 provided for each patient.

25 In the overall evaluation of the clinical signs designated as anticipated events, in those
26 volunteers who designated the severity as "severe", the total events were ranked in the following
27 order for the combined groups: elevated temperature above 100 °F. (21), pain at the injection site

(15), headaches (5), chills (4), muscular pain (4), discoloration (3), weakness (2), nausea (2), swelling (2), and redness (2).

Where the anticipated events were designated as “moderate”, the events were ranked as follows for the combined groups: temperature between 98.0 and 99.9 °F. (104), pain at the injection site (30), swelling (27), weakness (9), chills (8), headache (7), treatment groups collectively, there were 8/30 complete regressions, 6/30 that were reduced in size, 10/30 that were not changed in size, 2/30 that were enlarged and 4/30 that were lost to follow-up. In the control group, there were no regressions, no reductions in size, 2/3 that were not changed in size and 1/3 that was enlarged.

These studies show that while concentrations below 0.4 mg/ml are adequate, the volumes required for efficacy are subsequently higher. Therefore, the test material should be concentrated above 0.4 mg per milliliter in order to reduce the volume of intralesional injections. Since there were a number of complete regressions in the groups where the material was administered subcutaneously, both intralesional and subcutaneous administration separately, or in combination, are efficacious.

Example 4.

Clinical Toxicities of *P. acnes* in human subjects.

P. acnes, manufactured within the State of Florida (ImmunoMed Corporation) has been administered intravenously to 21 cancer patients in a completed Phase I study conducted under Florida law. The patients were comprised of 14 males and 7 females, age 38 to 73 years (median = 56). The dosage per injection ranged from 25 ug to 800 ug, and the total dosage ranged from a low of 50 ug to a high of 8525 ug.

A total of 256 injections were administered to these patients, and 44 were associated with toxicity (17.2%). Toxicities reported included chills (24/256 - 9.4%), fever (22/256 - 8.6%), nausea (10/256 = 3.9%), myalgia (4/256 - 1.6%), malaise (2/256 - 0.8%), and lightheadedness (2/256 - 0.8%). There was no injection site toxicity reported.

1 In another experiment with *P. acnes*, 3 healthy male volunteers were administered the
2 immunostimulant I.V.. Two received 0.1 mg (0.0012 mg/kg) and the third received 0.2 mg
3 (0.0023 mg/kg.). Fever, chills, malaise, lethargy, and slight muscle soreness were experienced by
4 all three individuals beginning 12-18 hours following injection. One individual, who received 0.2
5 mg, experience slight nausea without vomiting. Symptoms abated within 24 hours after onset. One
6 individual received 0.1 mg was administered a second injection of 0.1 mg 27 days after the first
7 injection. Only a slight fever (1°F. increase) was recorded with no other symptomatology.

8 Intralesional and subcutaneous injections of the test material have minimally associated
9 toxicities. Intravenous administration should have toxicities similar to those reported previously.

1 **We claim:**

- 2 1. A method of inducing the regression of dermal tumors in humans which comprises the step
3 of administering a bacterial product comprising heat-killed *P. acnes* bacteria selected from the
4 group consisting of *Propionibacterium acnes*, *Propionibacterium avidum*, *Propionibacterium*
5 *lymphophilum*, *Propionibacterium granulosum*, *Corynebacterium parvum* or *Arachnia*
6 *propionica*.
- 7 2. The method of claim 1 wherein the bacterial product that is administered comprises heat-
8 killed *Propionibacterium acnes*
- 9 3. The method of claim 1, wherein the method induces the regression of dermal tumors caused
10 by the human papilloma virus.
- 11 4. The method of claim 1, wherein the bacterial product further comprises an anesthetic.
- 12 5. The method of claim 4, wherein the anesthetic is selected from the group consisting of
13 aminoamides and aminoesters.
- 14 6. The method of claim 4, wherein the anesthetic is lidocaine.
- 15 7. The method of claim 1, wherein the bacterial product further comprises carriers and fillers.
- 16 8. The method of claim 7, wherein the carriers are selected from the group consisting of sugars
17 including but not limited to lactose, saccharose, mannitol, sorbitol, and cellulose preparations.
- 18 9. The method of claim 7, wherein the carriers are selected from the group consisting of
19 amino acids including but not limited to glycine.
- 20 10. The method of claim 7, wherein the fillers are selected from the group consisting of starch
21 pastes that use corn, wheat, rice or potato starch, gelatin, methylcellulose,
22 hydroxypropylmethylcellulose, and sodium carboxymethylcellulose.
- 23 11. The method of claim 1, wherein the bacteria are heat-killed by the process of heating the *P.*
24 *acnes* in a water bath at 74 ° C to 90 ° C for 60 to 90 minutes.
- 25 12. The method of claim 1, wherein the bacterial product is suspended in a saline solution.

- 1 13. The method of claim 12, wherein the saline solution comprises sodium chloride in dI water.
- 2 14. The method of claim 12, wherein the saline solution comprises sodium chloride in a buffer.
- 3 15. The method of claim 14, wherein the buffer is selected from the group consisting of alkaline
4 phosphates and alkaline citrates.
- 5 16. The method of claim 1, wherein the bacterial product is administered intralesionally.
- 6 17. The method of claim 1, wherein the bacterial product is administered subcutaneously.
- 7 18. The method of claim 1, wherein the bacterial product is administered preferably at .001 to 5
8 mg per dosage.
- 9 19. The method of claim 1, wherein the bacterial product is administered more preferably at
10 .005 to 2.5 mg per dosage.
- 11 20. The method of claim 1, wherein the bacterial product is administered most preferably at .01
12 to 1 mg per dosage.
- 13 21. A method of treating viral infections of the respiratory tract in humans which comprises the
14 step of administering a bacterial product comprising heat-killed *P. acnes* bacteria selected from the
15 group consisting of *Propionibacterium acnes*, *Propionibacterium avidum*, *Propionibacterium*
16 *lymphophilum*, *Propionibacterium granulosum*, *Cornynebacterium parvum* or *Arachnia*
17 *propionica*.
- 18 22. The method of claim 21 wherein the bacterial product comprises heat-killed
19 *Propionibacterium acnes*.
- 20 23. The method of claim 21, wherein the bacterial product further comprises carriers and
21 fillers.
- 22 24. The method of claim 23, wherein the carriers are selected from the group consisting of
23 sugars including but not limited to lactose, saccharose, mannitol, sorbitol, and cellulose preparations.
- 24 25. The method of claim 23, wherein the carriers are selected from the group consisting of
25 amino acids including but not limited to glycine.

- 1 26. The method of claim 23, wherein the fillers are selected from the group consisting of starch
2 pastes that use corn, wheat, rice or potato starch, gelatin, methylcellulose, hydroxypropylmethyl-
3 cellulose, and sodium carboxymethylcellulose.
- 4 27. The method of claim 21, wherein the bacteria are heat-killed by the process of heating the
5 *P. acnes* in a water bath at 74 °C to 90 °C for 60 to 90 minutes.
- 6 28. The method of claim 21, wherein the bacterial product is suspended in a saline solution.
- 7 29. The method of claim 28, wherein the saline solution comprises salts selected from the group
8 consisting of alkaline phosphates and alkaline citrates.
- 9 30. The method of claim 21, wherein the bacterial product is administered orally.
- 10 31. The method of claim 21, where the bacterial product is administered with a natural flavoring
11 or artificial flavoring.
- 12 32. The method of claim 21, wherein the bacterial product is administered preferably at .1 to 10
13 mg per dosage.
- 14 33. The method of claim 21, wherein the bacterial product is administered more preferably at
15 0.5 to 5 mg per dosage.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/28361

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 45/00

US CL : 424/282.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/282.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,746,511 A (KOBATAKE ET AL) 24 May 1988 (24/5/88), see entire document, especially column 8, lines 65-69 and column 13, line 61.	1-33
Y	US 4,479,935 A (METIANU ET AL) 30 October 1984 (30/10/84), see entire document, especially column 1, lines 58-64.	1-33

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Further documents are listed in the continuation of Box C.

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See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*&* document member of the same patent family
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Date of the actual completion of the international search 20 DECEMBER 2000	Date of mailing of the international search report 22 MAR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer FREDERICK KRASS Telephone No. (703) 308-1235